ABSTRACT

THE IMPORTANCE OF PRE- AND POSTNATAL THERMAL CONDITIONS IN DETERMINING GROWTH TRAJECTORIES IN THREE VIVIPAROUS GRASSLAND SNAKES

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In many reptile species, the onset of reproductive maturity is determined by size rather than age. Rapid growth during the first year may therefore promote population growth by shortening generation time and increasing the probability of survival to reproduction. Patterns of neonatal growth were observed over five years in three sympatric grassland snakes at Potawatomi Woods in Northern Illinois. Growth of Dekay's Brownsnakes (*Storeria dekayi*), Red-bellied Snakes (*S. occipitomaculata*) and Common Gartersnakes (*Thamnophis sirtalis*) varied in parallel among years such that neonates achieved 20-44% greater snout-vent length (SVL) in warmer years (mean April 1 thru September 30 temperature) than in cooler years ($F_{1,10} = 45.29$, $P < 0.001$). Variation in expected SVL on October 1 was better explained by mean April-May temperatures (partial $\eta^2 = 0.77$) than by June-July (partial $\eta^2 = 0.49$) or August-September temperatures (partial $\eta^2 = 0.48$), despite the fact that year-to-year variation in mean temperature was greatest for June-July (range in mean temperature among years = 4.1 °C for June-July vs. 3.2 °C for April-May and 1.8 °C for August-September). I interpret this result as evidence of the greater importance of environmental temperature on timing of ovulation (during April-May) than on embryonic development (during June-July), possibly because females can transcend year-to-year variation in temperature during gestation through

behavioral thermoregulation. To test this, I conducted an enclosure experiment in which thermoregulatory opportunities were manipulated. Individual enclosures were used to house 25 wild-caught gravid *S. dekayi* females divided among two temperature treatments. Females with greater opportunities to thermoregulate (Sun treatment) had parturition dates significantly earlier than females with restricted opportunities to thermoregulate (Shade treatment) ($F_{1,14} = 25.22$, $P < 0.001$). Together, field and experimental data suggest that females may be able to behaviorally compensate for lower temperatures during gestation, whereas lower temperatures in spring (April-May) may delay ovulation, resulting in smaller neonates by October 1, and potentially reducing population growth rates.

Key Words: Gestation; Growth; Ovulation; Parturition; *Storeria dekayi*; *Storeria occipitomaculata*; *Thamnophis sirtalis*; Thermoregulation; Viviparity

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THE IMPORTANCE OF PRE- AND POSTNATAL THERMAL CONDITIONS IN DETERMINING GROWTH TRAJECTORIES IN THREE VIVIPAROUS GRASSLAND SNAKES

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DEDICATION

In loving memory of my grandparents, Leeland “Lee” and “Erma” Gibbs, who would have wholehearted supported my affinity for reptilian ecology, despite their undoubted bewilderment over its development. For my parents, Roy and Dorothy O’Brien, without whose love and support this accomplishment would not have been possible.

TABLE OF CONTENTS

Page

LIST OF TABLES……... vii

LIST OF APPENDICES…….. viii

LIST OF FIGURES…….. ix

INTRODUCTION…… 1

Chapter

MATERIALS and METHODS…… 5

Field Studies: Neonatal Growth Trajectories Across Years and Among Species……. 5

Thermal Enclosure Experiment: Effect of Restricted Thermoregulatory Opportunities…… 8

RESULTS……. 15

Field Studies: Neonatal Growth Trajectories Across Years and Among Species……. 15

Thermal Enclosure Experiment: Effect of Restricted Thermoregulatory Opportunities…… 23

Chapter Page

DISCUSSION…….. 26

Importance of Temperatures on Neonatal Growth Trajectories……... 26

Growth Trajectories and Reproductive Maturity……. 28

Effects of Restricted Thermoregulatory Opportunities on Parturition Date…….. 30

Insight into the Evolution to Viviparity…… 33

Implications for Conservation Management…… 34

Concerning Climate Change…… 35

LITERATURE CITED…… 37

APPENDICES……. 41

LIST OF TABLES

Table Page

1. Number of neonate captures at Potawatomi Woods, DeKalb, Illinois by year and species.……. 16
2. Regression equations for 2009-2013 neonatal growth trajectories from ANCOVAs of SVL by year with Capture day of year for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata*, and expected SVL on October 1 for each year and species…… 20
3. Tests for covariate effects of mean seasonal temperature on expected SVL on October 1 and corresponding effect sizes…… 23
4. Summary of average daily mean, minimum, and maximum soil and shaded air temperatures from June 19 thru August 15 2013 for the Sun treatment, Shade treatment, Potawatomi Woods Forest Preserve, and Nachusa Grasslands…….. 24
5. Most advanced stages of embryonic development reached based on morphological characteristics from dissection of pre-partum females following Zehr (1962)…….. 25

LIST OF APPENDICES

Appendix Page

A. GROWTH TRAJECTORIES: EQUALITY OF SLOPES AND ELEVATIONS…… 41

B. EXPECTED SVL ON OCTOBER 1…… 43

C. PARTURITION DATES……. 45

D. LITTER EFFECTS…….. 47

LIST OF FIGURES

Figures Page

1. GoogleEarth image of Potawatomi Woods Forest Preserve, DeKalb County, Illinois showing the approximate position of cover board locations with wet sedge meadow habitat circled in yellow……... 6
2. Plot of SVL vs. DOY for *S. dekayi* captures in 2011. Neonates appear as a distinct size class……... 7
3. Individual nylon mesh pop-up laundry hamper enclosure…… 9
4. Hamper enclosure containing natural substrate, vegetation, water dish and ceramic tile platform to provide retreat sites and opportunities to bask…….. 9
5. Layout of hamper enclosures behind greenhouse for Sun and Shade treatments……. 10
6. Ultrasonography of female *T. sirtalis* using VisualSonics Vevo770 Small Animal Imaging System……... 12
7. Ultrasonography image of enlarged follicles of gravid female *S. dekayi* with no sign of embryonic development…….. 13
8. Ultrasonography image of gestating *S. dekayi* showing spinal column of two developing embryos……. 13

Figures Page

9. Year-specific neonatal growth trajectories for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata*…….. 17

10. Expected snout-vent length on October 1 for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata* from 2009–2013……. 21

11. Mean monthly ambient temperatures for DeKalb County, Illinois, for 2009-2013. April-May, June-July, and August-September are highlighted based on their biological significance to pre- and postnatal growth…… 22

12. Relationship between expected SVL on October 1 and mean April-May temperature from 2009-2013 for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata* 29

INTRODUCTION

For many reptiles, reproductive maturity is strongly correlated with body size, such that more rapid growth during the first year of life results in earlier maturity (Brewster et al., 2013). Therefore, growth during the first year of life can influence population dynamics by shortening generation time and increasing survival to reproduction (Gibbons et al., 1981). Reduced growth rates of hatchling Eastern Collared Lizards (*Crotaphytus collaris*) resulted in fewer females reaching sexual maturity within one year (Brewster et al., 2013). Additionally, first year growth can impact lifetime growth rates through a "silver-spoon effect" in which individuals that are able to grow more quickly in their first year experience higher lifelong growth rates (Le Galliard et al., 2005; Baron et al., 2010; Henaff et al., 2013). For example, cohorts of Brown Water Pythons (*Liasis fuscus*) with rapid first year growth (born in years with high prey abundance) continued to grow faster across years relative to cohorts with slower first year growth (born in years with low prey abundance) (Madsen and Shine, 2000). These results suggest neonatal growth rates can have impacts on long-term growth and lifetime reproductive output, important components of population dynamics (Oli and Dobson, 2003).

Postnatal growth trajectories of viviparous reptiles can be influenced by prenatal events through timing of birth and size at birth (Wapstra et al., 2009; While et al., 2009;

Baron et al., 2010). Offspring mass and offspring body condition of Meadow Vipers (*Vipera ursinii ursinii*) increased with earlier parturition dates, resulting in faster postnatal growth rates (Baron et al., 2010). For many viviparous reptiles, temperature can influence the duration of gestation (Lourdais et al., 2004, Wapstra et al., 2009) and allow the prediction of timing of birth (Blanchard and Blanchard, 1940). Viviparous Northern Watersnakes (*Nerodia sipedon*) gave birth 56 days earlier in warmer treatments (25.2 °C with continuous access to a heating rock) than those from cooler treatments (20.9 °C with access to a heating rock limited to two hours per day) (Blouin-Demers et al., 2000). Local ambient temperature during gestation (mean of daily maximums) was the key predictor of date of birth for two wild populations of viviparous Ocellated Skink (*Niveoscincus ocellatus*) (Cadby et al., 2010).

Environmental temperature can impact offspring growth and survival through an effect on timing of birth (Monasterio et al., 2013). Oviparous Common Wall Lizard (*Podarcis muralis*) eggs incubated at lower temperatures (2.3°C cooler) hatched later, yielding smaller hatchlings with reduced hatchling survival compared to hatchlings incubated at warmer temperatures (Henanff et al., 2013). Additionally, *P. muralis* hatchlings from the cooler incubation treatment that did survive their first winter were significantly smaller at one year of age, indicating long-term fitness impacts from environmental temperatures during embryonic development (Henanff et al., 2013). These results show how offspring fitness (growth and survival) can be influenced by parturition date (Wapstra et al., 2009; Du et al., 2012), with potential impacts on population dynamics through survival to first reproduction and age at reproductive maturity (Gibbons et al., 1981).

With timing of birth correlated with offspring growth, survival and potentially age of reproductive maturity, the question arises of whether timing of mating (possibly related to

timing of emergence and therefore also temperature dependent) has an impact on timing of birth. In the Common Gartersnake (*Thamnophis sirtalis*), date of mating (up to three weeks apart) did not impact timing of birth across four years (Blanchard and Blanchard, 1940). In contrast, parturition dates did vary across years in response to mean ambient temperatures, such that a decrease in mean temperature of 1 °F (mean of daily extremes from May 1 thru July 31) delayed parturition by approximately 4.5 days (Blanchard and Blanchard, 1940).

It seems clear that first year growth trajectories can be affected by timing of birth, with the potential to impact offspring survival and lifetime reproductive output. Therefore, factors influencing timing of birth, particularly temperature, are worth study. Understanding the driving micro-climatic factors that impact reproductive timing and success in temperate viviparous snakes may provide finer scale resolution for estimating important snake life history traits. This is important for reptile species of conservation concern because their generally limited dispersal abilities makes them particularly vulnerable to microclimate changes in small habitat patches. With embryonic development in many reptiles showing phenotypic responses to environmental temperatures (Blouin-Demers et al., 2000; Lourdais et al., 2004; Cadby at al., 2010), issues associated with climate change present an additional challenge and the need for further research into the sensitivity of pre- and postnatal growth to temperature variation. Understanding the effects of microclimate on date of birth and first year growth can provide conservation biologists with indices for estimating population dynamics in response to local environmental fluctuations, and may be applied to the management of other temperate ectothermic reptile species (Beaupre and Douglas, 2009).

Here, I report evidence that prenatal thermal conditions influence neonatal growth trajectories in wild populations of three sympatric grassland species in Northern Illinois. Field

observations suggest that variation in mean April-May temperatures (timing of ovulation and early gestation) had a greater effect on year-to-year variation in neonatal snout-vent length (SVL) on October 1 than variation in mean June-July temperature (majority of gestation) or mean August-September temperature (parturition and postnatal growth) across all three species. I hypothesize that females may compensate for variation in June-July temperatures through behavioral thermoregulation and report the results of an outdoor enclosure experiment with *Storeria dekayi* (Dekay's Brownsnake) designed to test this hypothesis.

MATERIALS AND METHODS

Field Studies: Neonatal Growth Trajectories Across Years and Among Species

From April 2009 through October 2013, a capture-mark-recapture study of Dekay's Brownsnake (*Storeria dekayi*), Red-bellied Snake (*Storeria occipitomaculata*), and Common Gartersnake (*Thamnophis sirtalis*) was conducted at Potawatomi Woods Forest Preserve in northern DeKalb County, Illinois. Field work was focused in a seasonally flooded wet sedge meadow (approximately 3.4 ha) and adjacent old field habitats (Fig. 1). From 2009 to 2012, 33 cover boards measuring ca. 60 x 90 x 1 cm and made of used rubber conveyor belt were deployed. During the 2013 field season, the number of cover boards was increased to 41. Cover boards were checked approximately weekly from late March through October, and snakes beneath them were hand captured. Snakes were classified by sex, measured to obtain mass and snout-vent length (SVL), and individually marked by ventral scale clipping (Fitch, 1987). Snakes were processed and released where captured, usually within 5 to 10 minutes.

I identified neonates (animals captured prior to their first hibernation) as a distinct age class by plotting SVL against day of year (DOY) separately for each year and species (Fig. 2). After selecting just the neonatal size class, I used SPSS Software (SPSS 22.0, IBM) to run an analysis of covariance with SVL as dependent variable, year as factor, and capture DOY as covariate to test for (1) equality of slopes and (2) equality of intercepts among years within each species and to generate equations relating SVL to DOY for each year and species combination. To compare year-to-year variation in neonatal growth trajectories, day of year

274 (October 1) was chosen to represent the approximate end of the growing season prior to first hibernation, and

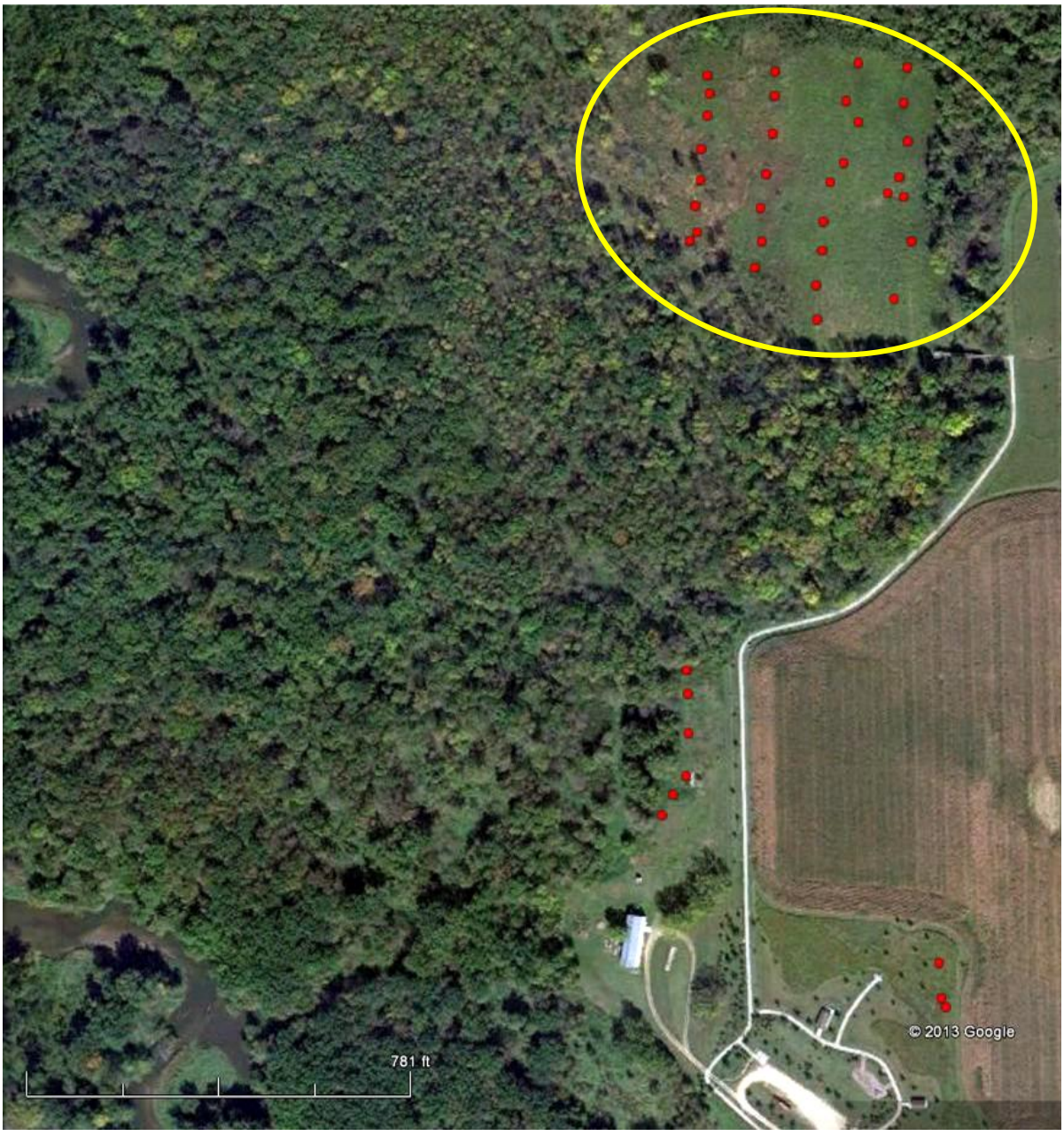


Figure 1: GoogleEarth image of Potawatomi Woods Forest Preserve, DeKalb County, Illinois showing the approximate position of cover board locations (red) with wet sedge meadow habitat circled in yellow.

an expected SVL on October 1 was calculated for each year for all three species from their respective regression equations.

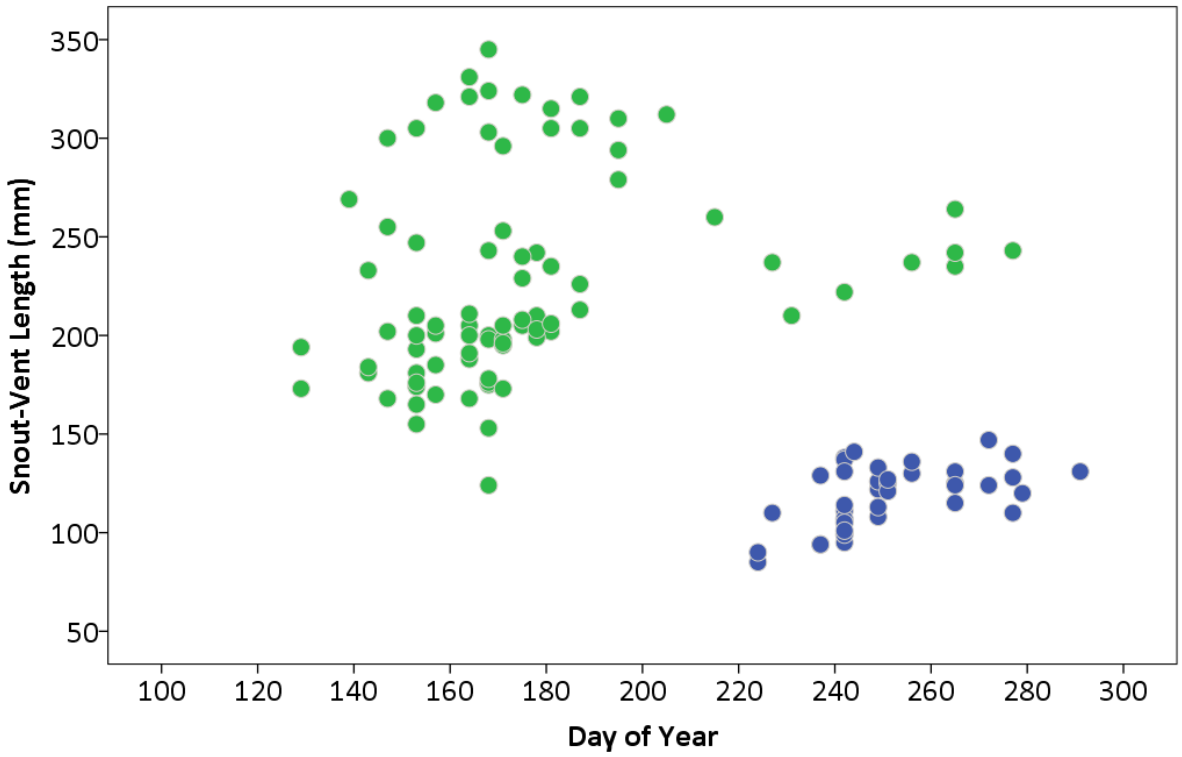


Figure 2: Plot of SVL vs. DOY for *S. dekayi* captures in 2011. Neonates (blue) appear as a distinct size class (*n* =38).

To identify possible causes of year-to-year variation in neonatal growth trajectories, annual ambient temperature data were compiled from the NIU Department of Geography Cooperative Weather Station located in DeKalb County. Annual mean April 1 thru September 30 temperatures were calculated from the midpoint of daily maximum and daily minimum temperatures. To better understand how temperature influenced expected SVL on October 1, I

chose three specific time periods within the April-September active season due to their biological significance to pre- and postnatal growth. These included April-May which is associated with ovulation and early gestation, June-July which represents the majority of gestation, and August-September when parturition and postnatal growth occur.

I used analysis of covariance, with species as a factor, to test whether expected SVL on October 1 covaried with mean April-September temperature. I then repeated this analysis using mean April-May, June-July, and August-September temperature, generating estimates of effect size (partial η^2) to assess which time period had the greatest influence on expected SVL on October 1. Here, partial η^2 represents the proportion of variation in expected SVL on October 1 explained by mean temperature during a specific time period after removing the variation attributable to species (Richardson, 2011).

Thermal Enclosure Experiment: Effect of Restricted Thermoregulatory Opportunities

Gravid *S. dekayi* were collected between May 24 and July 4, 2013 from Potawatomi Woods (n = 13) and Nachusa Grasslands (n = 12), a restored prairie located 45 km away in Lee and Ogle Counties. Females were housed in individual outdoor enclosures consisting of nylon mesh pop-up laundry hampers 71cm in height by 46 cm in diameter (Fig. 3). Hampers were buried to a depth of 15 cm and filled with natural substrate (soil, stones, vegetation). Each hamper contained a water dish and ceramic tile platform to provide retreat sites and opportunities to bask (Fig. 4). Water and food (earthworms) were provided ad libitum.

Figure 3: Individual nylon mesh pop-up laundry hamper enclosure.

Figure 4: Hamper enclosure containing natural substrate, vegetation, water dish and ceramic tile platform to provide retreat sites and opportunities to bask.

Enclosures were divided into two treatment types, Shade (mostly shaded, n = 12) and Sun (partially shaded, n = 13). Sun and Shade enclosure treatments were surrounded by fencing to prevent predation and provide structural support for the hampers (Fig. 5), and were located within a restricted-access area on the Northern Illinois University campus. Females from each site were divided between the two treatment types and randomly assigned to an enclosure. Females were checked daily for parturition and neonates were counted and measured to obtain mass and SVL within 24 hours of birth. Females were released at their site of capture within a few days of parturition.

Figure 5: Layout of hamper enclosures behind greenhouse for Sun (A) and Shade (B) treatments.

To characterize differences in thermal conditions between treatments, soil and shaded air temperatures were recorded every hour using Thermocron iButton temperature loggers.

Loggers were buried at a depth of 10 cm (one per enclosure) or mounted on stakes at a height of 1.5 m and enclosed within a radiation shield constructed of PVC pipe capped with a T-fitting to allow for air flow (two per treatment). Average daily mean, average daily minimum, and average daily maximum temperatures were calculated from hourly temperatures for each logger between June 19 and August 15 (the first parturition date for a female within an enclosure). I ran an analysis of variance with average daily mean logger temperature as dependent variable and treatment as factor for both soil and shaded air temperature loggers (Sun treatment: soil $n = 12$, air $n = 2$, Shade treatment: soil $n = 12$, air $n = 2$). To compare thermal conditions of experimental treatments with field conditions, soil and shaded air temperatures were also recorded every hour at Potawatomi Woods (logger stations: soil $n = 3$, air $n = 3$) and Nachusa Grasslands (logger stations: soil $n = 7$, air $n = 7$).

Delayed development may have contributed to the death of four females in the Shade treatment between August 26 and September 2. Consequently, ultrasonography (Sacchi et al., 2012) using a VisualSonics Vevo770 Small Animal Imaging System was used to assess offspring development of the twelve remaining pre-partum females on September 6 (Fig. 6). Females that were not gravid ($n = 1$), contained only enlarged follicles that showed no evidence of development ($n = 2$; Fig. 7), or showed evidence of minimal development ($n = 2$) were released at their site of capture. Females showing embryonic development (embryonic spinal column, embryonic heartbeat, blood flow; Fig. 8) were returned to the Sun treatment ($n = 1$) or moved from the Shade to the Sun treatment ($n = 6$). Three of these females died between September 13 and September 26. I dissected all seven pre-partum females that died during the experiment and calculated an expected date of birth for all but one from the most advanced stage of embryonic development reached based on morphological characteristics

identified by Zehr (1962). One female containing only enlarged follicles could not be assigned an expected date of birth.

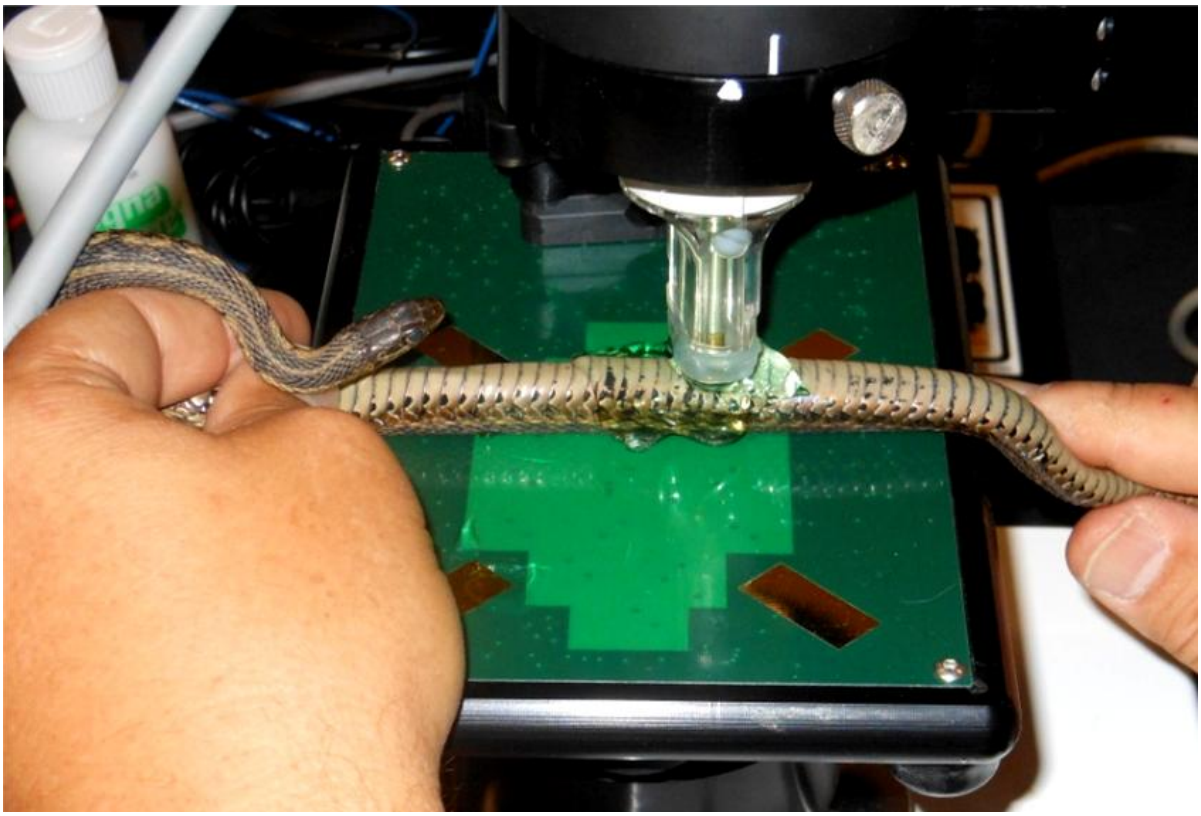


Figure 6: Ultrasonography of female *T. sirtalis* using VisualSonics Vevo770 Small Animal Imaging System.

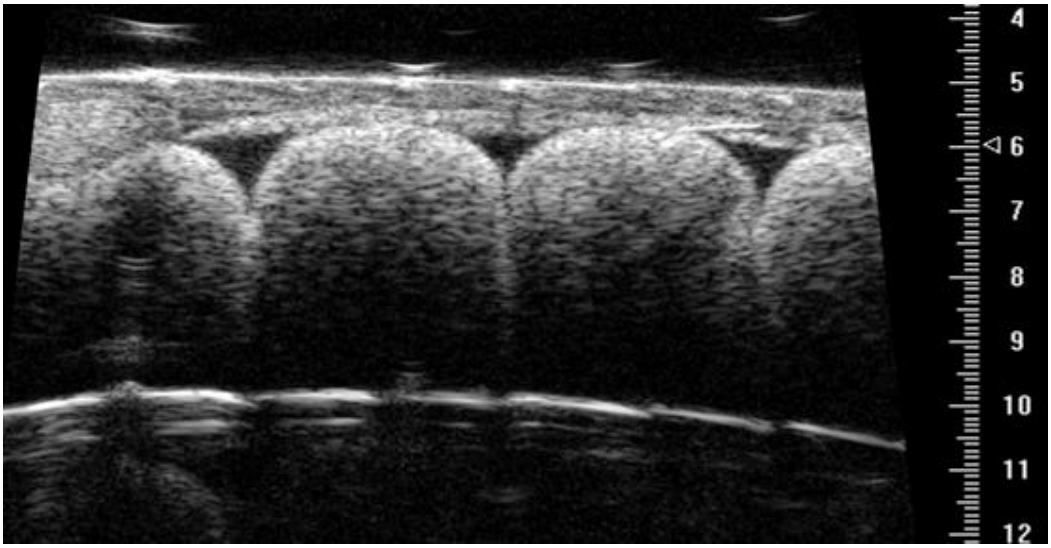


Figure 7: Ultrasonography image of enlarged follicles of gravid female *S. dekayi* with no sign of embryonic development.

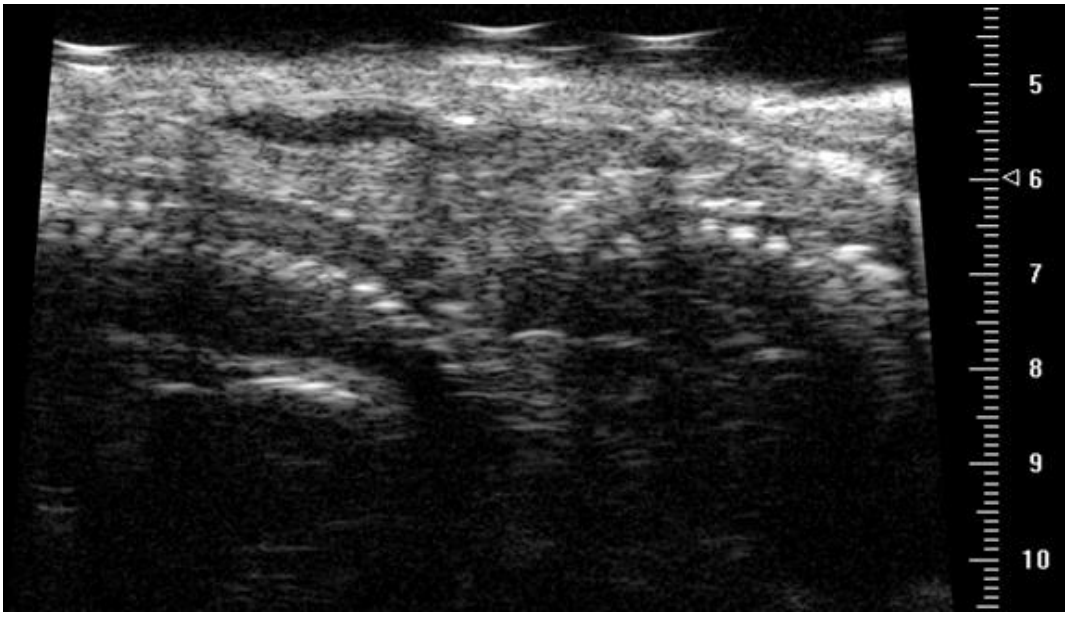


Figure 8: Ultrasonography image of gestating *S. dekayi* showing spinal column of two developing embryos.

To test for an effect of treatment on parturition date, I pooled observed parturition dates (n = 12) with expected parturition dates estimated from dissections (n = 6), and ran an analysis of variance with observed + expected birth DOY as dependent variable and treatment and location (female site of capture) as factors. I also tested for an effect of treatment on offspring SVL and offspring mass using analyses of variance with litter mean SVL or litter mean mass as dependent variables and treatment and location as factors. I tested for an effect of treatment on litter size, using analysis of covariance with litter size as dependent variable, female SVL as covariate, and treatment and location as factors. I tested for an effect of date of capture on parturition date using analysis of covariance with birth DOY as dependent variable, capture DOY as covariate, and treatment and location as factors.

RESULTS

Field Studies: Neonatal Growth Trajectories Across Years and Among Species

Neonate captures numbered 22 to 49 per year for *T. sirtalis*, 8 to 38 per year for *S. dekayi*, and 1 to 53 per year for *S. occipitomaculata* (Table 1). Analysis of covariance revealed that the relationship between SVL and DOY did not differ significantly in slope, but did differ significantly in elevation among years in *T. sirtalis* and *S. dekayi* (*T. sirtalis* equality of slopes: $F_{4,156} = 2.13$, $P = 0.079$, *S. dekayi* equality of slopes: $F_{4,93} = 0.22$, $P = 0.929$; *T. sirtalis* equality of elevations: $F_{4,160} = 23.13$, $P < 0.001$, *S. dekayi*; equality of elevations: $F_{4,97} = 27.83$, $P < 0.001$; Fig. 9, Appendix A). The slope of the relationship between SVL and DOY differed significantly among years in *S. occipitomaculata* (equality of slopes: $F_{3,100} = 7.27$, $P < 0.001$; Fig. 9, Appendix A). As a result, expected SVL on October 1 varied among years in parallel across species (Table 2, Fig. 10). Expected SVL on October 1 was greatest in 2010 and 2012 and least in 2009, 2011, and 2013 for all three species (Fig. 10).

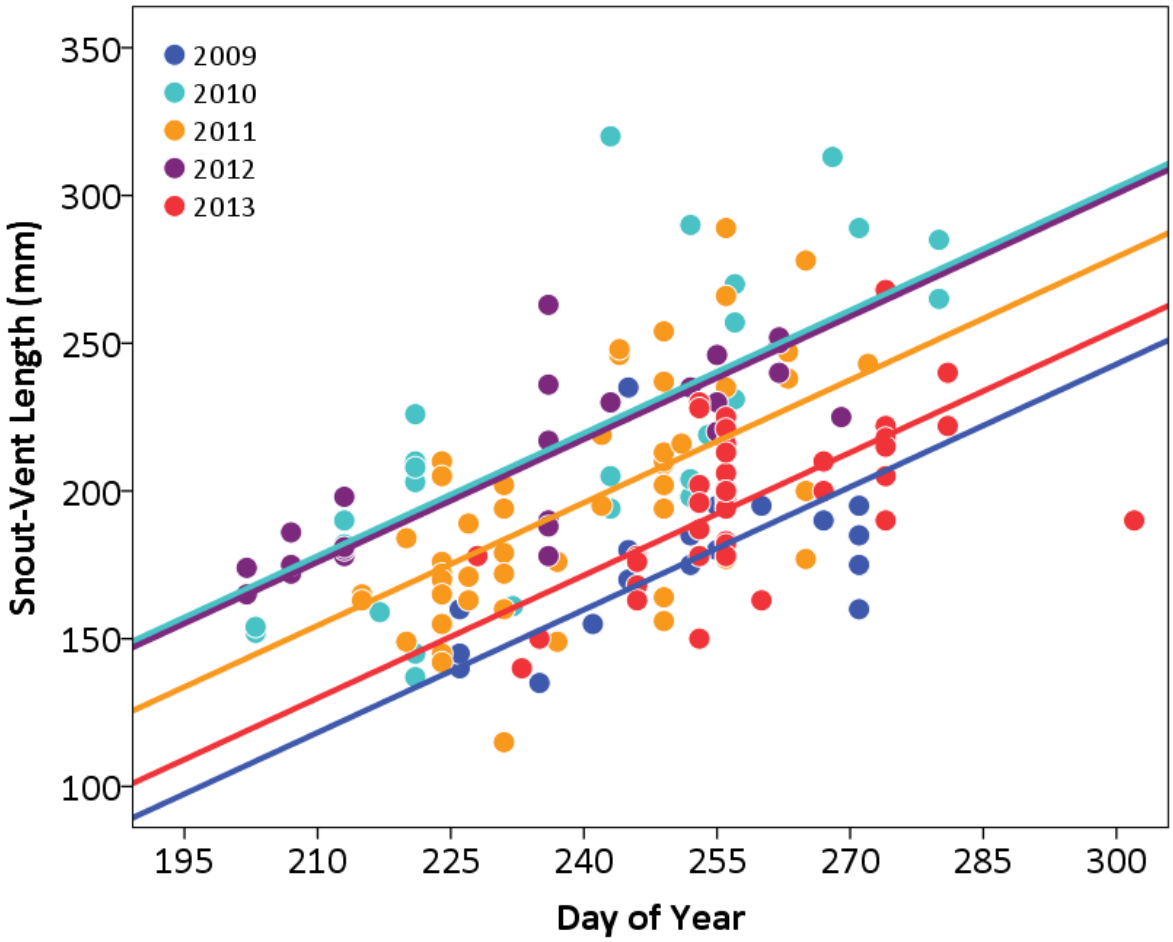
Mean April 1 thru September 30 temperature varied 2.6 °C between the warmest and coolest years during the five year study period. Expected SVL on October 1 differed among species ($F_{2,10} = 184.27$, $P < 0.001$) and covaried with mean April-September temperature ($F_{1,10} = 45.29$, $P < 0.001$) such that *S. dekayi*, *T. sirtalis*, and *S. occipitomaculata* neonates were 20-44% longer on October 1 in warmer years than in cooler years. Mean April-May, June-July, and August-September temperatures varied among years by 3.2 °C, 4.1 °C, and 1.8 °C, respectively (Fig. 11). Estimates of effect size showed that variation in mean April-May

temperatures best explained the variation in expected SVL on October 1 (April-May: $F_{1,10}$ = 33.83, $P < 0.001$, partial $\eta^2 = 0.77$; Table 3, Appendix B). Every degree increase in mean April-May temperature resulted in an approximately 8 mm increase in expected SVL on October 1 across all three species. Mean June-July and August-September temperatures also showed a significant association with expected SVL on October 1 but with smaller effect sizes (June-July: $F_{1,10} = 9.47$, $P = 0.012$, partial $\eta^2 = 0.49$; August-September: $F_{1,10} = 9.09$, $P = 0.013$, partial $\eta^2 = 0.48$; Table 3, Appendix B).

Table 1: Number of neonate captures (total captures) at Potawatomi Woods, DeKalb, Illinois by year and species.

Year	*T. sirtalis*	*S. dekayi*	*S. occipotimaculata*	Totals
2009	22 (121)	18 (52)	12 (41)	52 (214)
2010	26 (149)	10 (65)	1 (32)	37 (246)
2011	49 (150)	38 (120)	29 (59)	116 (329)
2012	24 (75)	8 (27)	14 (31)	46 (133)
2013	45 (145)	29 (130)	53 (119)	127 (394)
Totals	166 (640)	103 (394)	109 (282)	378 (1,316)

A. *T. sirtalis* Neonates



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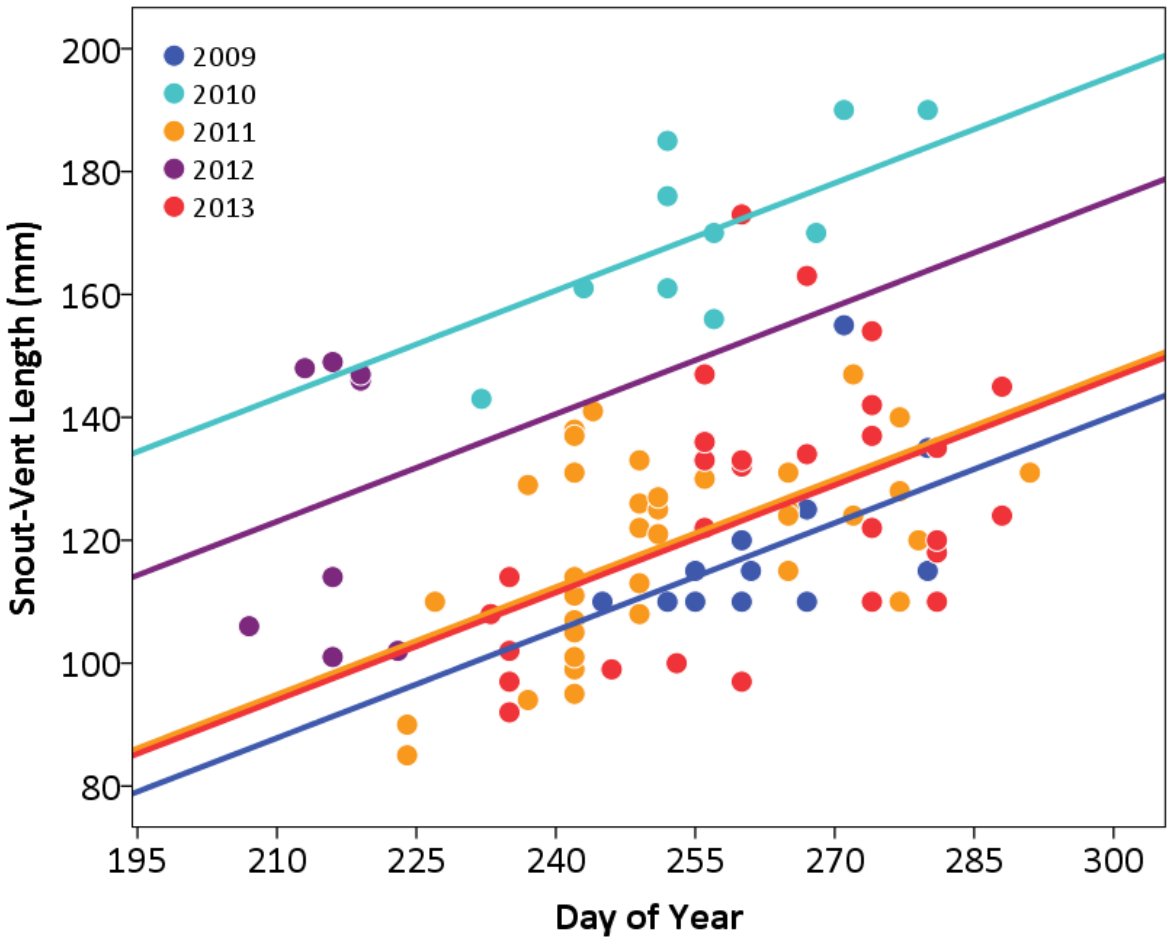
B. *S. dekayi* Neonates



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C. *S. occipitomaculata* Neonates

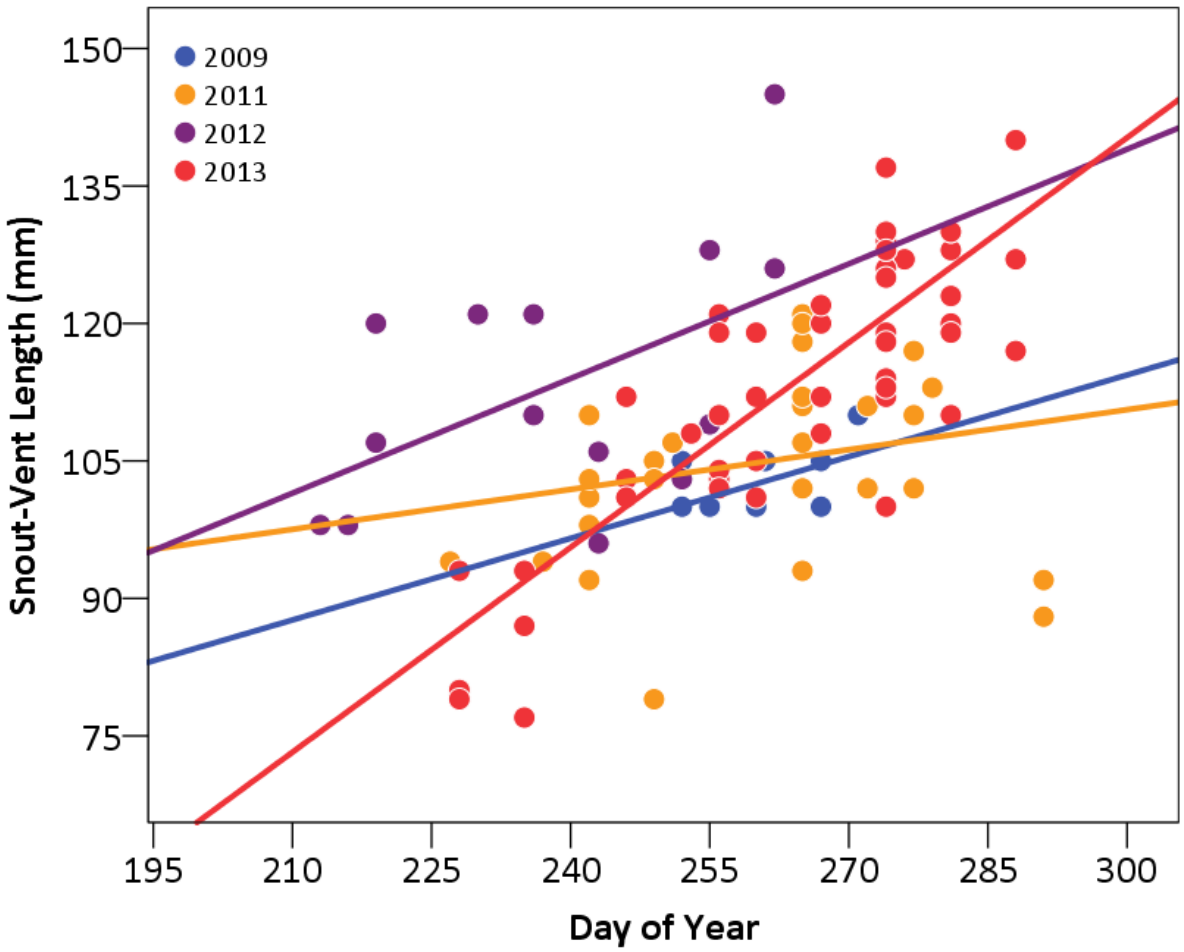

Figure 9: Year-specific neonatal growth trajectories for *T. sirtalis* (A), *S. dekayi* (B), and *S. occipitomaculata* (C).

Table 2: Regression equations for 2009-2013 neonatal growth trajectories from ANCOVAs of SVL by year with Capture day of year for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata*, and expected SVL on October 1 for each year and species.

Species	Year	Regression Equation	Expected SVL on October 1 (mm)
T. sirtalis	2009	-172.584 + 1.385 × DOY	206.9
	2010	-113.175 + 1.385 × DOY	266.3
	2011	-136.358 + 1.385 × DOY	243.1
	2012	-114.934 + 1.385 × DOY	264.6
	2013	-160.927 + 1.385 × DOY	218.6
S. dekayi	2009	-34.593 + 0.583 × DOY	125.1
	2010	20.728 + 0.583 × DOY	180.5
	2011	-27.869 + 0.583 × DOY	131.9
	2012	0.632 + 0.583 × DOY	160.4
	2013	-28.426 + 0.583 × DOY	131.3
S. occipitomaculata	2009	25.278 + 0.297 × DOY	106.7
	2011	67.091 + 0.145 × DOY	106.8
	2012	13.914 + 0.417 × DOY	128.2
	2013	-83.229 + 0.745 × DOY	120.9

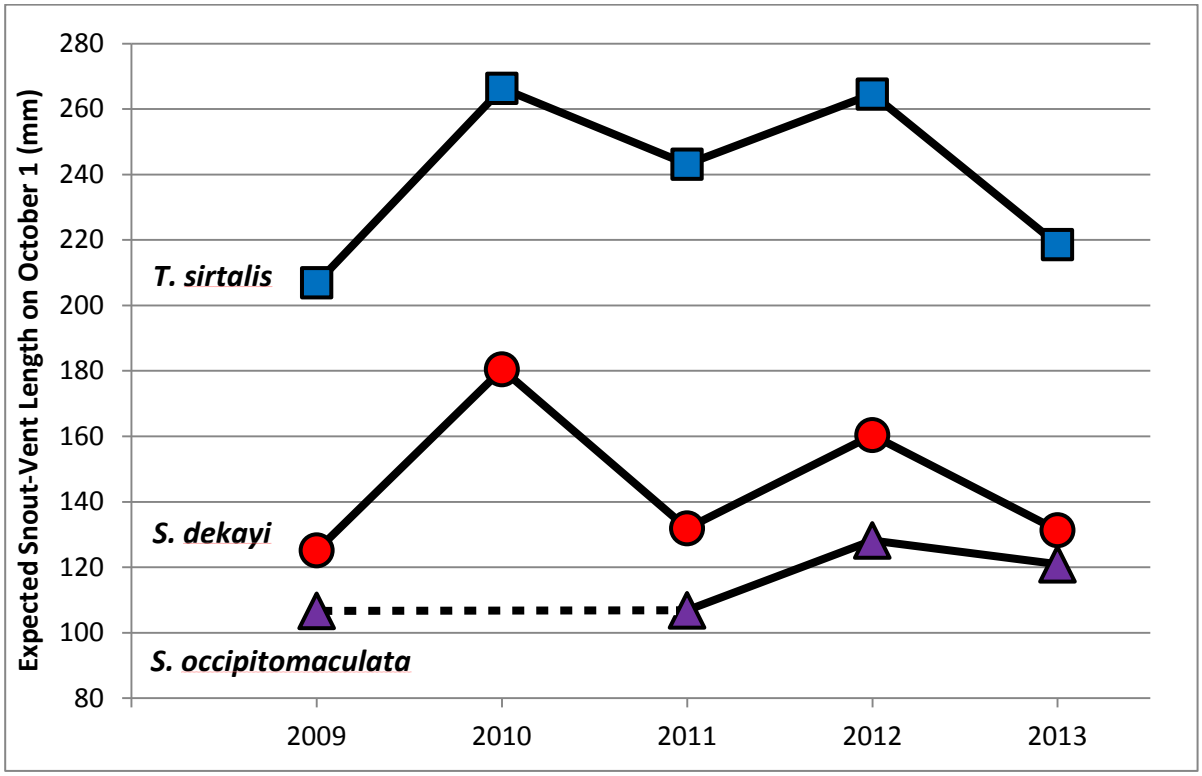

Figure 10: Expected snout-vent length on October 1 for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata* from 2009–2013. No data was available in 2010 for *S. occipitomaculata* due to low capture numbers, indicated by dashed line between 2009 and 2011.

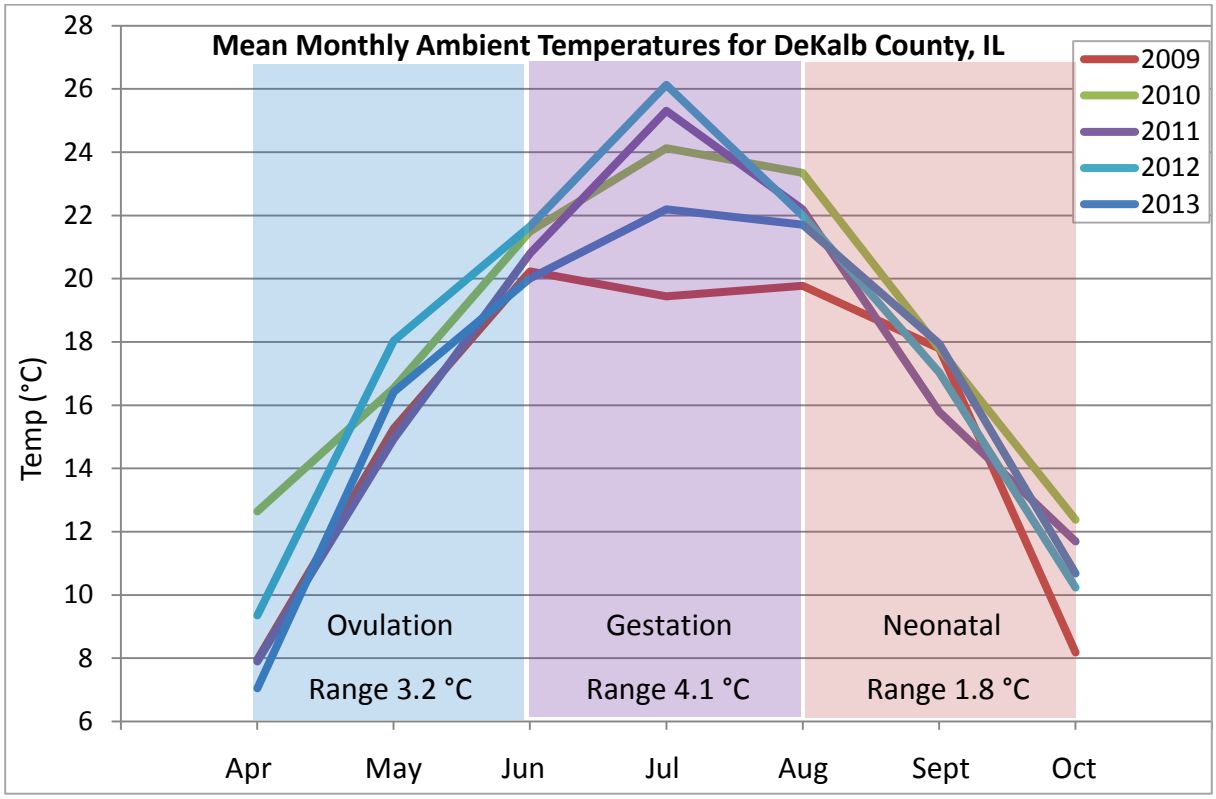

Figure 11: Mean monthly ambient temperatures for DeKalb County, Illinois, for 2009-2013. April-May, June-July, and August-September are highlighted based on their biological significance to pre- and postnatal growth.

Table 3: Tests for covariate effects of mean seasonal temperature on expected SVL on October 1 and corresponding effect sizes (partial η^2).

Associated Biological Process	Covariate	Significance	Partial η^2
Ovulation	Mean April-May temperature	$F_{1,10} = 33.829$, $P < 0.001$	0.772
Gestation	Mean June-July temperature	$F_{1,10} = 9.471$, $P = 0.012$	0.486
Neonatal growth	Mean August-September temperature	$F_{1,10} = 9.087$, $P = 0.013$	0.476

Thermal Enclosure Experiment: Effect of Restricted Thermoregulatory Opportunities

Between June 19 and August 15 2013, mean soil temperatures differed between treatments (Sun = 21.2 °C, Shade = 19.5 °C; $F_{1,22} = 438.89$, $P < 0.001$). Mean shaded air temperatures did not differ between treatments (Sun = 21.7 °C, Shade = 21.5 °C; $F_{1,2} = 4.69$, $P = 0.163$). On sunny days, Sun treatment enclosures received between 4 to 5 hours of direct sunlight, whereas Shade treatment enclosures received less than 30 minutes. With significantly warmer soil temperatures and greater availability of sunlight in the Sun than the Shade treatment, thermoregulatory opportunities of gestating females differed considerably between treatments.

Mean shaded air temperatures of Sun and Shade treatments and mean maximum air temperature of the Sun treatment were similar to those at Potawatomi Woods and Nachusa Grasslands (Table 4). However, mean maximum air temperature of the Shade treatment was lower than that of the Sun treatment and at field sites (Table 4). Mean minimum air

temperature was similar between Sun and Shade treatments, but higher than at field sites (Table 4). Mean, minimum, and maximum soil temperatures were consistently lower at Potawatomi Woods than at Nachusa Grasslands, and this difference was paralleled by differences between Shade and Sun treatments (Table 4).

Table 4: Summary of average daily mean, minimum, and maximum soil and shaded air temperatures (and standard deviations) from June 19 thru August 15 2013 for the Sun treatment, Shade treatment, Potawatomi Woods Forest Preserve, and Nachusa Grasslands.

Logger position	Location	# of Loggers	Average daily mean temperature (°C)	Average daily minimum temperature (°C)	Average daily maximum temperature (°C)
Soil	Sun	12	21.2 (0.16)	20.2 (0.22)	22.6 (0.36)
	Shade	12	19.5 (0.24)	18.8 (0.0.21)	20.3 (0.28)
	Potawatomi	3	19.8 (0.53)	18.7 (0.38)	21.1 (0.98)
	Nachusa	7	21.7 (1.26)	19.6 (0.91)	24.1 (1.84)
Shaded Air	Sun	2	21.7 (0.06)	16.5 (0.05)	28.6 (0.42)
	Shade	2	21.5 (0.11)	16.7 (0.22)	26.6 (0.13)
	Potawatomi	3	20.7 (0.27)	12.5 (0.42)	29.7 (0.36)
	Nachusa	7	21.8 (0.28)	14.1 (0.76)	29.3 (0.27)

Nine females gave birth in the Sun and three females in the Shade treatment. Expected parturition date was estimated by dissection for one additional Sun treatment female and five additional Shade treatment females (Table 5). Mean observed + expected parturition date of Sun treatment females (August 26, $n = 10$) differed significantly from that of Shade treatment females (September 21, $n = 8$; $F_{1,14} = 25.22$, $P < 0.001$; Appendix C). Similar results were obtained when only observed parturition dates were used; mean parturition date was August

22 for Sun treatment females (n = 9) and September 18 for Shade treatment females (n = 3; $F_{1,8}$ = 88.32, P < 0.001; Appendix C).

Table 5: Most advanced stages of embryonic development reached based on morphological characteristics from dissection of pre-partum females (n = 6) following Zehr (1962).

Zehr Stage	Treatment	Distinguishing morphological characteristics	Estimated days until birth
36	3 Shade, 1 Sun	Pigmentation pattern, heart and brain not visible, scalation on body, around mouth, snout and eyes, some hemipenes everted	9
32	2 Shade	No pigmentation pattern, heart visible, brain visible, tongue visible, scalation on body but not visible on mouth, no everted hemipenes	28

Mean offspring SVL did not differ between treatments (Sun = 76.9 mm, Shade = 72.4 mm; $F_{1,8}$ = 3.54, P = 0.096; Appendix D). Mean offspring mass did not differ between treatments (Sun = 0.34 g, Shade = 0.32 g; $F_{1,8}$ = 0.72, P = 0.420; Appendix D). Mean litter size of Sun treatment females (n = 13.2) and Shade treatment females (n = 9.3) did not differ ($F_{1,7}$ = 0.01, P = 0.934; Appendix D). Date of capture had no effect on parturition date ($F_{6,3}$ = 0.36, P = 0.868; Appendix D).

DISCUSSION

Importance of Temperatures on Neonatal Growth Trajectories

S. dekayi, *T. sirtalis*, and *S. occipitomaculata* showed parallel patterns of variation in neonatal growth trajectories across five years at Potawatomi Woods, such that neonates were 20-44% longer on October 1 in warmer years (mean April-September temperatures) than in cooler years (Fig. 10). This variation was best explained by variation in mean April-May temperatures (partial $\eta^2 = 0.77$), suggesting the importance of environmental temperature during ovulation and early embryogenesis on neonatal growth trajectories. Sensitivity of embryonic development to environmental conditions appears to vary based on stage of development (Lourdais et al., 2004). Early embryonic development has been shown to have cross-generational effects in some species, such that conditions experienced during early stages of embryonic development can influence offspring phenotype for multiple generations (Burton and Metcalfe, 2014).

Between 2009 and 2013, mean temperature varied the most during June-July (4.1 °C), when the majority of gestation occurs. Possibly, females can more easily compensate for variation in June-July vs. April-May temperature through behavioral thermoregulation because of the generally higher temperatures in June-July. During the five year study period, mean April-May temperatures were 8.2 to 11.6 °C cooler than mean June-July temperatures. Neonatal growth trajectories may be influenced by other factors determined by spring temperatures, such as time of emergence from hibernation (possibly through an effect on

parturition date) or time spent foraging (if more time is spent foraging during spring, there is less time available for thermoregulation) in addition to timing of ovulation. Further research is needed to determine if these indirect effects of spring temperature may have contributed to the results observed here.

Mean year-to-year temperature variation was least during August-September, the period associated with postnatal growth. Neonatal growth rates were equal (equal slopes) across years for both *T. sirtalis* and *S. dekayi*, suggesting cohorts born following warm spring temperatures (2010 and 2012) achieved longer SVL on October 1 due to factors unrelated to late summer temperature. However, variation in postnatal growth rates has been documented in other reptile species (Bronikowski, 2000; Wapstra et al., 2009; While et al., 2009; Baron et al., 2010) possibly through variation in prey abundance and thermoregulatory opportunities. Juvenile European Common Lizards (*Zootoca vivipara*) from a low food treatment had reduced postnatal growth rates resulting in smaller body size and poorer body condition prior to hibernation than siblings from a high food treatment (Le Galliard et al., 2005). Limiting thermoregulatory opportunities of juvenile Eastern Collared Lizards (*C. collaris*) resulted in reduced growth rates compared to juveniles from an unrestricted treatment (Brewster et al., 2013). Postnatal growth rates can also be influenced by timing of birth through an effect on length of foraging season prior to hibernation.

Rapid postnatal growth may have long term impacts on fitness by maintaining faster growth rates throughout an organism's life (Madsen and Shine, 2000; Baron et al., 2010; Heneff et al., 2013). However, some species exhibit compensatory growth, such that offspring born smaller or with less initial growth go through a period of accelerated (catch-up) growth (Metcalfe and Monaghan, 2001). Juvenile Jacky Lizards (*Amphibolurus muricatus*) from a

low food treatment had accelerated growth during the following 6 months with abundant food, such that they caught up in size with high food treatment siblings (Radder et al., 2007). Further research is needed on post-hibernation growth to distinguish if either of these alternative growth patterns occurs among one of the grassland snake species studied here.

Growth Trajectories and Reproductive Maturity

Between 2009 and 2013, every degree increase in mean Apr-May temperature resulted in an 8 mm increase in expected SVL on October 1 across species (Fig. 12). Consequently, attainment of reproductive maturity may be shortened from two years to one year for cohorts following a warm spring. In fact, expected SVL on October 1 of *S. dekayi* neonates approached the minimum SVL of reproductively mature males (SVL = 175 mm based on presence of sperm in cloacal smears, unpublished observation) following the warmest springs (2010: expected SVL =180.5 mm, 2012: expected SVL = 160.4 mm; Table 2). Among six neonatal males captured in 2010, four approached and two exceeded the minimum SVL of reproductive maturity (capture dates: August 31 to September 28, SVL: 156-190 mm). At present, it is unknown if neonates reaching sizes of known reproductively mature snakes are capable of reproduction. Reproductive maturity is determined by age rather than size for some populations of western terrestrial Gartersnake (*Thamnophis elegans*) (Bronikowski and Arnold, 1999). Further collection of cloacal smears from neonates, especially prior to and upon emergence from hibernation would provide insight into whether reproductive maturity is strictly size dependent among these sympatric snake species.

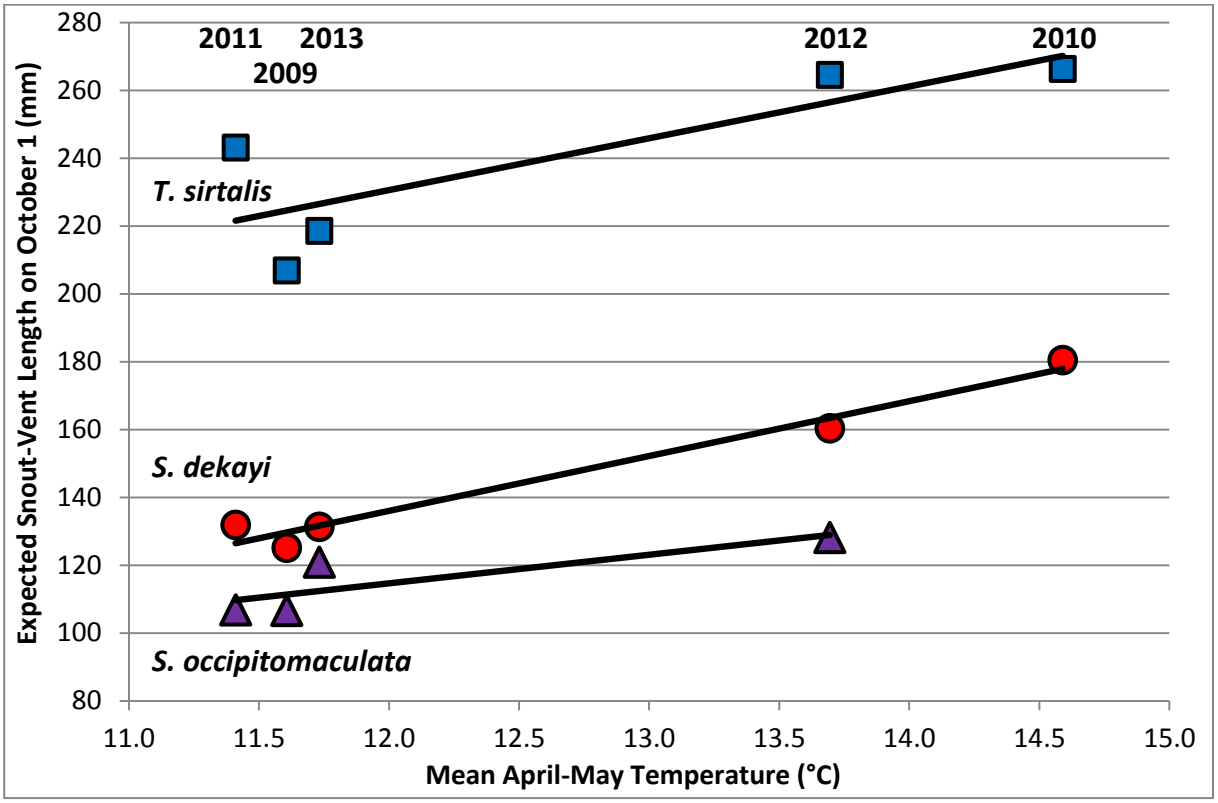

Figure 12: Relationship between expected SVL on October 1 and mean April-May temperature from 2009-2013 for *T. sirtalis*, *S. dekayi*, and *S. occipitomaculata* (year corresponding to mean temperature is listed above for reference). No data was available in 2010 for *S. occipitomaculata* due to lack of captures.

Effects of Restricted Thermoregulatory Opportunities on Parturition Date

To use results obtained from captive births to make inferences about natural *S. dekayi* populations, I sought to keep gravid females in enclosures that closely mimicked natural conditions. Time spent in captivity among gravid *S. dekayi* housed indoors (in plastic terraria) negatively impacted offspring size and body condition (King, 1993). Outdoor enclosures have been used to maintain gravid female snakes of several species until parturition and have the advantage over indoor housing in that snakes are exposed to natural variation in temperature, sunlight, and other factors that may promote successful reproduction (Farrell et al., 2009; Pilgrim et al., 2011). Mesh laundry hampers permitted airflow and water drainage while the open top provided direct access to sunlight. Further indication that environmental conditions within the enclosures simulated natural conditions was abundant vegetation growth within the enclosures. Additionally, mean shaded air temperatures within the enclosures were within the range of those at both field sites (Table 4).

Shade and Sun enclosures were grouped together (Fig. 5), a necessity imposed by the limited availability of sunnier vs. shadier locations within restricted-access outdoor areas. Therefore, my enclosure design is open to the criticism of pseudoreplication (Hurlbert, 1984). However, Sun and Shade treatments were located close together (within 20 m). Sun and Shade treatments were also similar with respect to other environmental variables (substrate type, soil moisture, vegetation characteristics, and ambient noise).

Mean soil temperature differed significantly between treatments, and represented

differences observed between field sites, with higher mean soil temperatures in the Sun treatment (21.2 °C) and at Nachusa Grasslands (21.7 °C) than in the Shade treatment (19.5 °C) or at Potawatomi Woods (19.8 °C; Table 4). Although I cannot be sure when parturition occurred in the field, the difference in first appearance of *S. dekayi* neonates at Nachusa Grasslands (August 1 2013, SVL = 108 mm) and at Potawatomi Woods (August 21 2013, SVL = 76 mm) may represent variation in parturition dates through an effect on timing of ovulation due to differences in thermal conditions similar to those between Sun and Shade treatments. However, larger SVL of Nachusa neonate indicates postnatal growth may have occurred following an earlier parturition date. Although there were no site effects on parturition date, litter mean SVL, litter mean mass, or mean litter size (Appendix D), further research is needed to determine differences in parturition date and neonatal growth trajectories between field sites.

Maternal thermoregulation by viviparous reptiles may buffer the thermal environment of developing embryos from external temperature variation and extremes in harsh temperate environments (Shine, 1995). Thermoregulatory opportunities of Shade treatment females were limited as indicated by mean soil temperature (19.5 vs. 21.2 °C in Shade vs. Sun enclosures) and extent of direct sunlight (<0.5 vs. 4.5 hr per day in Shade vs. Sun enclosures). Limiting thermoregulatory opportunities of Shade treatment females during gestation presumably affected timing of birth through an effect on the rate of embryonic development. Temperature during gestation affects rate of embryonic development in many species (Burton and Metcalfe, 2014). For reptiles in particular, this effect of temperature can influence parturition date (Cadby et al., 2010, Le Galliard et al., 2010) and offspring phenotype (Lourdais et al., 2004; Heneff et al., 2013; Monasterio et al., 2013). Female viviparous Ocellated Skinks (*N.*

ocellatus) with limited thermoregulatory opportunities (4 hours access to heat lamp) had later parturition dates, smaller offspring at birth and following hibernation, and reduced offspring survival compared to females maintained in a non-limited thermal treatment (10 hours access to heat lamp) (Wapstra et al., 2009).

Although there was no significant difference in mean offspring size at birth between treatments, there may still be differences in offspring fitness as a result of differences in parturition dates. Offspring born earlier (as seen in the Sun treatment) have a longer foraging season than offspring born later (as seen in the Shade treatment), resulting in larger size at the onset of hibernation. Postnatal growth trajectories influence offspring survival through an effect of offspring size at time of hibernation (Bronikowski, 2000). Greater postnatal growth of Sun treatment offspring (due to earlier parturition dates) may also result in the attainment of reproductive maturity earlier than offspring born to Shade treatment females.

Differing opportunities for thermoregulation resulted in behaviorial differences between Shade and Sun treatment females. Pairs of Sun and Shade treatment females ($n = 8$) were observed simultaneously every 30 minutes for 24 hours during July using programmable cameras. During daytime hours, Shade treatment females were visible in 90% of photos compared to 30% for Sun treatment females (paired $t = 14.55$, $P < 0.001$). These results demonstrate that females alter their thermoregulatory behavior in response to their thermal environment. However, increased exposure of Shade treatment females was not sufficient to compensate for differences in thermal conditions (soil temperature and sunlight) between treatments. Additionally, there are costs associated with thermoregulation, such as increased risk of predation and less time available for foraging (Brewster et al., 2013).

Insight into the Evolution to Viviparity

In cold climates, proportionally more squamates have evolved to viviparity from oviparous ancestors, supporting the hypothesis that maternal egg retention had a selective advantage over earlier oviposition through increased offspring survival (the cold-climate hypothesis,Tinkle and Gibbons, 1977; Shine, 1995). However, viviparity is also common outside of cold climates, and other hypotheses suggest alternative adaptive advantages of viviparity beyond buffering developing embryos from lethally cold temperatures associated with species latitude and elevation range limits (Shine, 1995). In particular, my results demonstrate a potential advantage to viviparity in allowing females to behaviorally compensate for year-to-year variation in temperature during gestation. Temperate species offer a unique opportunity to observe phenotypic responses of offspring to maternal environmental variation while simultaneously considering evolutionary adaptive mechanisms that may be at work (Shine, 2014).

During the enclosure experiment, females in the Shade treatment altered their thermoregulatory behavior for the perceived benefit of an optimal thermal embryonic environment (possibly through an effect on parturition date). This observation suggests female thermoregulation for earlier parturition date is adaptive in *S. dekayi*. As discussed previously, earlier parturition date may result in larger postnatal growth trajectories, which may enhance offspring fitness through effects on overwinter survival, growth rate, age at maturity, and lifetime reproductive output, all of which are adaptive advantages outside the

scope of the cold-climate hypothesis.

Implications for Conservation Management

Conservation managers are challenged with preserving species and habitat diversity, including a complex matrix of biotic and abiotic interactions. Reptiles have served as indicator species of ecosystem function and effective habitat management because of their responsiveness to environmental variation and unique life history traits (Webb and Shine, 2000; Ficheux et al., 2014). Annual variation in field metabolic rates is correlated with prey (small mammal) abundance for Timber Rattlesnakes (*Crotalus horridus*), resulting in trophic level responses to fire suppression in the Ozark Mountains (Beaupre and Douglas, 2009). This has led to a new forest management and restoration plan to improve ecosystem quality for wildlife (Beaupre and Douglas, 2009).

Results from my field observations and enclosure experiment suggest that neonatal growth trajectories may also provide a measure of habitat quality and management success. Neonatal growth trajectories can vary in response to numerous environmental factors including spring temperatures and thermoregulatory opportunities of gestating females (results presented here), as well as prey abundance and quality (Bronikowski, 2000; Brewster et al., 2013). Therefore growth trajectories are responsive to issues concerning conservation managers, such as factors associated with climate change (temperature and precipitation), habitat alteration (fire and grazing management), and trophic interactions (Sexton et al., 1992).

Growth during the first year of life may also provide a useful metric for the management of species of conservation concern (Madsen and Shine, 2000), because it contributes to population growth rates. Greater first year growth can increase population growth rates by increasing the probability of offspring survival to first reproduction and shortening generation time (Gibbons et al., 1981). My results suggest it may be possible to promote rapid population growth of species of concern by managing for desirable thermal conditions during time periods critical to pre- and postnatal development.

Concerning Climate Change

My field observations and enclosure experiment are particularly relevant to impacts associated with climate change because the most notable changes occurring in North America are the advancement and warming of spring and lengthening of summer (Bale et al., 2002; Parmesan, 2006). Many species have experienced a shift in one or more life history event associated with these changes in temperate climates (Bale et al., 2002; Yang and Rudolf, 2010). Some temperate reptiles may be positively affected by warmer springs and longer summers (Le Galliard et al., 2010). However, reptiles with temperature dependent sex determination could be negatively impacted (Walther et al., 2002; Refsnider et al., 2013).

My results suggest that *T. sirtalis, S. dekayi* and *S. occipitomaculata* may benefit from warmer spring temperatures (earlier parturition date) and longer summers (more time for foraging before hibernation) through effects on offspring fitness (increased survival, reduced age at maturity). However, earlier springs and longer summers may result in variation in annual precipitation (Parmesan, 2006), which may have negative impacts through effects on

prey availability (Shine and Madsen, 2000) or habitat quality (Seigel and Fitch, 1985). Annual variation in clutch sizes of oviparous Ring-necked Snakes (*Diadophis punctatus*) correlates with fall precipitation, such that clutch sizes are smaller in years with less rainfall (Seigel and Fitch, 1985). Hatchling Iberian Emerald Lizards (*Lacerta schreiberi*) incubated at high temperature treatments were larger in high moisture treatments than in low moisture treatments (Monasterio et al., 2013). Postnatal growth trajectories of viviparous European Common lizards (*Z. vivipara*) varied across years due to variation in early neonatal growth rates (first month of growth after birth) which increased with greater rainfall during mid gestation and decreased with greater rainfall after birth (Le Galliard et al., 2010). Further research is needed to determine what direct or indirect impacts precipitation may have on postnatal growth trajectories in these three viviparous grassland snake species.

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APPENDIX A

GROWTH TRAJECTORIES: EQUALITY OF SLOPES AND ELEVATIONS

Appendix A. Tables of ANCOVAs of SVL by year with capture day of year for *T. sirtalis* (A), *S. dekayi* (B), and *S. occipitomaculata* (C).

Dependent Variable: SVL

Source	Sum of Squares	df	Mean Square	F	Sig.
A. *T. sirtalis*					
Test for equality of slopes (Year*DOY)					
Year	4281.069	4	1070.267	1.590	0.180
DOY	74168.480	1	74168.480	110.167	<0.001
Year * DOY	5739.600	4	1434.900	2.131	0.079
Error	105024.803	156	673.236		
Total	230096.000	165			
Test for equality of elevations (Year) and effect of covariate (DOY)					
DOY	95685.139	1	95685.139	138.218	<0.001
Year	64038.253	4	16009.563	23.126	<0.001
Error	110764.404	160	692.278		
Total	230096.000	165			
B. *S. dekayi*					
Test for equality of slopes (Year*DOY)					
Year	109.393	4	27.348	0.114	0.977
DOY	1240.492	1	1240.492	5.168	0.025
Year * DOY	207.162	4	51.791	0.216	0.929
Error	22323.120	93	240.034		
Total	52837.049	102			
Test for equality of elevations (Year) and effect of covariate (DOY)					
DOY	7268.341	1	7268.341	31.293	<0.001
Year	25859.028	4	6464.757	27.833	<0.001
Error	22530.282	97	232.271		
Total	52837.049	102			
C. *S. occipitomaculata*					
Test for equality of slopes (Year*DOY)					
Year	1696.414	3	565.471	6.857	<0.001
DOY	993.397	1	993.397	12.046	0.001
Year * DOY	1799.740	3	599.913	7.274	<0.001
Error	8246.986	100	82.470		
Total	19359.213	107			

APPENDIX B

EXPECTED SVL ON OCTOBER 1

Appendix B. Tables of 2009-2013 ANCOVA of expected SVL on October 1 by species with mean temperature.

Dependent Variable: Expected SVL on October 1

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta^2
April 1-Sept 30	4212.434	1	4212.434	44.919	<0.001	0.818
Species	37604.423	2	18802.212	200.498	<0.001	0.976
Error	937.775	10	93.778			
Total	45572.470	13				
April 1-May 31	4074.638	1	4074.638	37.883	<0.001	0.791
Species	36876.985	2	18438.493	171.430	<0.001	0.972
Error	1075.571	10	107.557			
Total	45572.470	13				
June 1-July 31	2450.184	1	2450.184	9.075	0.013	0.476
Species	39730.019	2	19865.009	73.573	<0.001	0.936
Error	2700.025	10	270.002			
Total	45572.470	13				
Aug 1-Sept 30	2464.976	1	2464.976	9.180	0.013	0.479
Species	37330.822	2	18665.411	69.511	<0.001	0.933
Error	2685.233	10	268.523			
Total	45572.470	13				

APPENDIX C

PARTURITION DATES

Appendix C. ANOVA table of observed + expected date of birth as dependent variable with treatment and location as factors, and ANOVA table of observed date of birth as dependent variable.

Dependent Variable: Observed + expected date of birth

Source	Sum of Squares	df	Mean Square	F	Sig.
Treatment	2651.089	1	2651.089	25.216	<0.001
Location	49.467	1	49.467	0.471	0.504
Treatment * Location	93.844	1	93.844	0.893	0.361
Error	1471.867	14	105.133		
Total	4764.444	17			

Dependent Variable: Observed date of birth

Treatment	1275.125	1	1275.125	88.320	<0.001
Location	48.347	1	48.347	3.349	0.105
Treatment * Location	2.347	1	2.347	0.163	0.697
Error	115.500	8	14.438		
Total	1795.667	11			

APPENDIX D

LITTER EFFECTS

Appendix D. ANOVA tables of mean offspring SVL and mean offspring mass as dependent variables and treatment and location as factors, ANCOVA table of litter size as dependent variable, with female SVL as covariate, and treatment and location as factors, and ANCOVA table of birth DOY as dependent variable, capture DOY as covariate, and treatment and location as factors.

Dependent Variable: Mean offspring SVL

Source	Sum of Squares	df	Mean Square	F	Sig.
Location	7.907	1	7.907	0.514	0.494
Treatment	54.496	1	54.496	3.545	0.096
Location * Treatment	12.756	1	12.756	0.830	0.389
Error	122.973	8	15.372		
Total	183.860	11			

Dependent Variable: Mean litter mass

Location	<0.001	1	<0.001	0.232	0.643
Treatment	0.001	1	0.001	0.722	0.420
Location * Treatment	<0.001	1	<0.001	0.254	0.628
Error	0.010	8	0.001		
Total	0.013	11			

Dependent Variable: Litter size

Female SVL	54.497	1	54.497	2.634	0.149
Treatment	0.154	1	0.154	0.007	0.934
Location	56.339	1	56.339	2.723	0.143
Treatment * Location	7.604	1	7.604	0.368	0.563
Error	144.837	7	20.691		
Total	474.250	11			

Dependent Variable: Birth DOY

Capture DOY	49.181	6	8.197	0.358	0.868
Location	22.533	1	22.533	0.984	0.394
Treatment	481.333	1	481.333	21.029	0.019
Error	68.667	3	22.889		
Total	1795.667	11			